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(54) Title: HUMANIZED ANTIBODIES

(57) **Abstract:** Humanized forms of mouse antibody 10D5 that retain the binding properties of mouse 10D5 are disclosed. Also disclosed are processes for making the humanized antibody, intermediates for making the humanized antibodies, including, nucleotide sequences, vectors, transformed host cells, and methods of using the humanized antibody to treat, prevent, alleviate, reverse, or otherwise ameliorate symptoms or pathology or both, that are associated with Down's syndrome or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy.

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HUMANIZED ANTIBODIES

This application claims priority of US 60/287,653, filed 2001 April 30, the entire contents of which are incorporated herein by reference.

The invention relates to humanized antibodies useful for treating and preventing human diseases associated with amyloid β (Aβ), such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy. Mouse monoclonal antibody 10D5 was raised by immunizing mice with human Aβ1-28, and has been widely used in analytical methods [*J. Neuropathol. Exper. Neurology 51*:76-83 (1992); *Nature 359*:325-327 (1992); *Neuroscience Lett. 172*:122-124 (1994); *Biochem. Biophys. Res. Commun. 200*:1598-1603 (1994); *J. Neuropathol. Exper. Neurology 53*:377-383 (1994); *Annals Neurology 37*:512-518 (1995); *Annals Neurology 41*:809-813 (1997); *J. Neuroimmunol. 88*:85-90 (1998); *J. Neuroimmunol. 95*:136-142 (1999)]. 10D5 has been shown to bind to the N-terminal region of Aβ and has affinity of approximately 43 pM for aggregated Aβ.

After 10D5 was administered to a group of 8.5 to 10.5 month-old heterozygous, transgenic PDAPP mice (APP^{V717F}) at a weekly intraperitoneal dose of about 10 mg/kg for six months, the mice had significantly reduced levels of Aβ1-42 in brain cortex. However, the 10D5 group did not have a significant reduction of total Aβ in any tissue, nor of Aβ1-42 in hippocampus or cerebellum [Bard, F., *et al., Nature Med.* 6:916-919 (2000); WO 00/72876 and WO 00/72880, 7 December, 2000]. It was asserted that amyloid plaques in the 10D5 group also reduced in number and appearance, with some evidence of cell-associated immunoreactivity.

Another study in WO 00/72876 and WO 00/72880 reported that administration of 10D5 to older mice for six months caused a significant reduction in amyloid β plaque burden. It was asserted that the antibody gained access to the central nervous system in sufficient amounts to "decorate" β-amyloid plaques. Finally, it was stated that mouse 10D5 induces phagocytosis of amyloid plaques in *in vitro* studies.

Methods for administering aggregated Aβ1-42 to provoke an immunologic response and reduced amyloid deposits are described in PCT publication WO99/27944, published 10 June 1999. The description postulates that full-length aggregated Aβ peptide would be a useful immunogen. The application also indicates that antibodies that bind to Aβ peptide could be used as alternate therapeutic agents. However, this appears

to be speculation since the supporting data reflect protocols that involve active immunization using, for example, Aβ1–42.

WO 99/60024, published 25 November 1999, is directed to methods for amyloid removal using anti-amyloid antibodies. The mechanism, however, is stated to utilize the ability of anti-Aβ antibodies to bind to pre-formed amyloid deposits (i.e. plaques) and result in subsequent microglial clearance of localized plaques. This mechanism was not proved *in vivo*. This publication further states that to be effective against Aβ plaques, anti-Aβ antibodies must be delivered directly to the brain, because antibodies cannot cross the blood brain barrier.

Queen, *et al.* describe methods of humanizing antibodies [*e.g.*, US Patent Nos. 5,585,089, 5,693,761, 5,693,762, 6,180,370].

Humanized forms of 10D5 are needed for use in humans having Down's syndrome, or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy (CAA). However, it is not known whether 10D5 can be humanized so that the humanized antibody retained the binding properties of the mouse antibody.

Summary of the Invention

This invention provides humanized forms of 10D5. These humanized antibodies have binding properties (affinity and epitope location) that are approximately the same as those of the mouse 10D5 antibody. The invention includes antibodies, single chain antibodies, and fragments thereof. The invention includes antibodies wherein the CDR are those of mouse monoclonal antibody 10D5 (sequences SEQ ID NO:1 through SEQ ID NO:6) and wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the variable regions have sequences comprising the CDR from mouse antibody 10D5 and specific human framework sequences (sequences SEQ ID NO:7 - SEQ ID NO:10), wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody 10D5. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the light chain is SEQ ID NO:11 and the heavy chain is SEQ ID NO:12.

Also part of the invention are polynucleotide sequences that encode the humanized antibodies or fragments thereof disclosed above, vectors comprising the polynucleotide sequences encoding the humanized antibodies or fragments thereof, host cells transformed with the vectors or incorporating the polynucleotides that express the humanized

5   antibodies or fragments thereof, pharmaceutical formulations of the humanized antibodies and fragments thereof disclosed herein, and methods of making and using the same.

Such humanized antibodies and fragments thereof are useful for, among other things, treating and preventing diseases and conditions characterized by Aβ plaques or Aβ toxicity in the brain, such as Alzheimer's disease, Down's syndrome, and cerebral

10   amyloid angiopathy in humans.

The invention also includes use of a humanized antibody of the present invention for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating, preventing, or reversing Alzheimer's disease, Down's syndrome, or cerebral amyloid angiopathy, or

15   to inhibit the formation of amyloid plaques or the effects of toxic soluble Aβ species in humans.

Detailed Description of the Invention

We have surprisingly found that humanized antibodies, wherein the CDRs originate from mouse monoclonal antibody 10D5 and the framework and other portions

20   of the antibodies originate from a human germ line, bind Aβ1-40 and Aβ1-42 with at least the affinity with which mouse 10D5 binds Aβ. Thus, we have a reasonable basis for believing that humanized antibodies of this specificity, modified to reduce their immunogenicity by converting them to a humanized form, offer the opportunity to treat, both prophylactically and therapeutically, conditions in humans that are associated with

25   formation of beta-amyloid plaques. These conditions include, as noted above, pre-clinical and clinical Alzheimer's, Down's syndrome, and pre-clinical and clinical cerebral amyloid angiopathy.

As used herein, the word "treat" includes therapeutic treatment, where a condition to be treated is already known to be present and prophylaxis - *i.e.*, prevention of, or

30   amelioration of, the possible future onset of a condition.

By "antibody" is meant a monoclonal antibody *per se*, or an immunologically effective fragment thereof, such as an Fab, Fab', or F(ab')$_2$ fragment thereof. In some contexts, herein, fragments will be mentioned specifically for emphasis; nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as the protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred. Antibodies are properly cross-linked via disulfide bonds, as is well known.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 3 or more amino acids.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N- terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat, et al., "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, et al., J. Mol. Biol. 196:901-917 (1987); Chothia, et al., Nature 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). A humanized immunoglobulin does not encompass a chimeric antibody, having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Humanized antibodies have at least three potential advantages over non-human and chimeric antibodies for use in human therapy:

1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC).

2) The human immune system should not recognize the framework or C region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody.

3) Injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected humanized antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The design of humanized immunoglobulins may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used

(acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing

non-human immunoglobulin (donor immunoglobulin):

(a) the amino acid in the human framework region of the acceptor

immunoglobulin is unusual for human immunoglobulin at that position, whereas the

corresponding amino acid in the donor immunoglobulin is typical for human

immunoglobulin at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms

(center-to-center) of any atom of a CDR amino acid in a three dimensional

immunoglobulin model [Queen, *et al.*, Proc. Natl. Acad. Sci. USA 86:10029-10033

(1989), and Co, et al., Proc. Natl. Acad. Sci. USA 88, 2869 (1991)]. When each of the

amino acid in the human framework region of the acceptor immunoglobulin and a

corresponding amino acid in the donor immunoglobulin is unusual for human

immunoglobulin at that position, such an amino acid is replaced by an amino acid typical

for human immunoglobulin at that position.

A preferred humanized antibody is a humanized form of mouse antibody 10D5.

The CDRs of humanized 10D5 have the following amino acid sequences:

light chain CDR1:
```
1               5                    10                   15
Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)
```

light chain CDR2:
```
1                    5
Lys Val Ser Asn Arg Phe Ser   (SEQ ID NO:2)
```

light chain CDR3:
```
1                    5
Phe Gln Gly Ser His Val Pro Leu Thr   (SEQ ID NO:3)
```

heavy chain CDR1:
```
1                    5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)
```

heavy chain CDR2:
```
1               5                    10                   15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)
```

and, heavy chain CDR3:
```
1               5                    10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr   (SEQ ID NO:6).
```

A preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK18 and J segment Jk4:

```
5     1                 5                      10                    15
      Asp Val Xaa Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Leu Gly

                        20                     25                    30
      Xaa Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Xaa His Ser
10
                        35                     40                    45
      Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser

                    50                  55                    60
15    Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

      65                    70                     75                    80
      Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

20                        85                     90                    95
      Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Phe Gln Gly

                        100                    105                   110
      Ser His Val Pro Leu Thr Phe Gly Xaa Gly Thr Lys Xaa Glu Ile Lys
25
      Arg                                                      (SEQ ID NO:7).
```

wherein:

Xaa at position 3 is Val or Leu;

Xaa at position 7 is Ser or Thr;

Xaa at position 14 is Thr or Ser;

Xaa at position 17 is Gln, Asp, or Asn;

Xaa at position 30 is Ile or Val;

Xaa at position 50 is Arg or Lys;

Xaa at position 88 is Val or Leu;

Xaa at position 105 is Gly or Ala; and

Xaa at position 109 is Val or Leu.

A preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-28 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity:

```
        1              5                    10                 15
        Xaa Xaa Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu

                       20                   25                 30
    5   Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

                       35                   40                 45
        Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

   10        50                   55                 60
        Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Xaa

             65                   70                 75                 80
        Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val
   15
                            85                   90                 95
        Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr

                       100                  105                110
   20   Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr

             115                  120
        Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser           (SEQ ID NO:8).
```

25   wherein:

   Xaa at position 1 is Gln or Glu;

   Xaa at position 2 is Val or Ala;

   Xaa at position 64 is Ser or Thr;

   Xaa at position 77 is Lys or Arg;

30   Xaa at position 78 is Ser or Thr;

   Xaa at position 83 is Thr or Ser;

   Xaa at position 84 is Met, Ile, or Leu;

   Xaa at position 86 is Asn, Ser, or Thr;

   Xaa at position 87 is Met, Val, or Leu; and

35   Xaa at position 118 is Leu or Ser.

A particularly preferred light chain variable region of a humanized antibody of the
present invention has the following amino acid sequence, in which the framework
originated from human germline Vk segment DPK18 and J segment Jk4:

```
40      1              5                    10                 15
        Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

                       20                   25                 30
        Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
   45
                       35                   40                 45
        Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
```

```
      50                      55                      60
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
```

5
```
  65                      70                      75                      80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
```

```
                  85                      90                      95
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
```
10
```
                 100                     105                     110
Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
```

15
```
Arg                                          (SEQ ID NO:9).
```

A particularly preferred heavy chain variable region of a humanized antibody of

20   the present invention has the following amino acid sequence, in which the framework

originated from human germline VH segment DP-28 and J segment JH4:

```
  1               5               10                      15
Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
```
25
```
              20                      25                      30
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
```

```
          35                      40                      45
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
```
30
```
      50                      55                      60
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
```

```
  65                      70                      75                      80
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
```
35
```
                  85                      90                      95
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
```

40
```
              100                     105                     110
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
```

```
          115                     120
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser            (SEQ ID NO:10).
```
45

A preferred light chain for a humanized antibody of the present invention has the

50   amino acid sequence:

```
  1               5               10                      15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
```

```
              20                      25                      30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
```
55

```
             35                      40                      45
        Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser


             50                      55                      60
  5     Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro


        65                      70                      75              80
        Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile


 10                          85                      90                  95
        Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly


                        100                     105                     110
        Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

 15
                    115                     120                     125
        Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu


                130                     135                     140
 20     Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe


        145                     150                     155                 160
        Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln


 25                         165                     170                 175
        Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser


                        180                     185                     190
        Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu

 30
                    195                     200                     205
        Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser


            210                     215
 35     Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys              (SEQ ID NO:11).
```

A preferred heavy chain for a humanized antibody of the present invention has the

amino acid sequence:

```
 40     1                   5                      10                      15
        Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu


                        20                      25                      30
        Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 45
                    35                      40                      45
        Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu


            50                      55                      60
 50     Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser


        65                      70                      75              80
        Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val


 55                         85                      90                  95
        Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr


                        100                     105                     110
        Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr

 60
```

```
                115                   120                   125
      Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly

            130                   135                   140
  5   Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly

      145                   150                   155                   160
      Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val

 10                     165                   170                   175
      Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

                  180                   185                   190
      Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val

 15               195                   200                   205
      Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val

            210                   215                   220
 20   Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys

      225                   230                   235                   240
      Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu

 25                     245                   250                   255
      Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr

                  260                   265                   270
      Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val

 30               275                   280                   285
      Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val

            290                   295                   300
 35   Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser

      305                   310                   315                   320
      Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu

 40                     325                   330                   335
      Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala

                  340                   345                   350
      Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro

 45               355                   360                   365
      Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln

            370                   375                   380
 50   Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala

      385                   390                   395                   400
      Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr

 55                     405                   410                   415
      Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

                  420                   425                   430
      Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

 60               435                   440                   445
      Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
```

450
Leu Ser Pro Gly Lys   (SEQ ID NO:12).

Other sequences are possible for the light and heavy chains for humanized 10D5.
The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least
one chain comprising one or more mouse complementarity determining regions
functionally joined to human framework region segments.

In another aspect, the present invention is directed to recombinant polynucleotides
encoding antibodies which, when expressed, comprise the heavy and light chain CDRs
from an antibody of the present invention. Exemplary polynucleotides, which on
expression code for the polypeptide chains comprising the heavy and light chain CDRs of
monoclonal antibody 10D5 are given herein. Due to codon degeneracy, other
polynucleotide sequences can be readily substituted for those sequences. Particularly
preferred polynucleotides of the present invention encode antibodies, which when
expressed, comprise the CDRs of SEQ ID NO:1 – SEQ ID NO:6, or any of the variable
regions of SEQ ID NO:7 – SEQ ID NO:10, or the light and heavy chains of SEQ ID
NO:11 and SEQ ID NO:12.

The polynucleotides will typically further include an expression control
polynucleotide sequence operably linked to the humanized immunoglobulin coding
sequences, including naturally-associated or heterologous promoter regions. Preferably,
the expression control sequences will be eukaryotic promoter systems in vectors capable
of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic
hosts may also be used. Once the vector has been incorporated into the appropriate host
cell line, the host cell is propagated under conditions suitable for expressing the
nucleotide sequences, and, as desired, the collection and purification of the light chains,
heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other
immunoglobulin forms may follow.

The nucleic acid sequences of the present invention capable of ultimately
expressing the desired humanized antibodies can be formed from a variety of different
polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and
components (e.g., V, J, D, and C regions), using any of a variety of well known

techniques. Joining appropriate genomic and synthetic sequences is a common method of
production, but cDNA sequences may also be utilized.

Below is a cDNA sequence (SEQ ID NO:17), from which the light chain having
the amino acid sequence of SEQ ID NO:19 may be expressed.

```
      ATGAAGTTGCCTGTTAGGCTGTTGGTACTGATGTTCTGGATTCCTGCTTCCAGCAGTGAT
    1 ---------+---------+---------+---------+---------+---------+ 60
      M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  -

      GTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACTCTTGGACAGCCAGCCTCCATC
   61 ---------+---------+---------+---------+---------+---------+ 120
      V  V  M  T  Q  S  P  L  S  L  P  V  T  L  G  Q  P  A  S  I  -

      TCTTGCAGATCTAGTCAGAACATTATACATAGTAATGGAAACACCTATTTAGAATGGTAC
  121 ---------+---------+---------+---------+---------+---------+ 180
      S  C  R  S  S  Q  N  I  I  H  S  N  G  N  T  Y  L  E  W  Y  -

      CTGCAGAAACCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTTCCAACCGATTTTCT
  181 ---------+---------+---------+---------+---------+---------+ 240
      L  Q  K  P  G  Q  S  P  R  L  L  I  Y  K  V  S  N  R  F  S  -

      GGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGC
  241 ---------+---------+---------+---------+---------+---------+ 300
      G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S  -

      AGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCGCTC
  301 ---------+---------+---------+---------+---------+---------+ 360
      R  V  E  A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P  L  -

      ACTTTCGGCGGAGGGACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTC
  361 ---------+---------+---------+---------+---------+---------+ 420
      T  F  G  G  G  T  K  V  E  I  K  R  T  V  A  A  P  S  V  F  -

      ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
  421 ---------+---------+---------+---------+---------+---------+ 480
      I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  -

      AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG
  481 ---------+---------+---------+---------+---------+---------+ 540
      N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  -

      GGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC
  541 ---------+---------+---------+---------+---------+---------+ 600
      G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S  -

      AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC
  601 ---------+---------+---------+---------+---------+---------+ 660
      S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  -

      ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT    (SEQ ID NO:17)
  661 ---------+---------+---------+---------+---------+---- 714
      T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  - (SEQ ID NO:19)
```

Below is a cDNA sequence (SEQ ID NO:18), from which the heavy chain having
the amino acid sequence of SEQ ID NO:20 may be expressed.

```
      ATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCCTGCATATGTCCTGTCCCAG
    1 ---------+---------+---------+---------+---------+---------+ 60
      M  D  R  L  T  S  S  F  L  L  L  I  V  P  A  Y  V  L  S  Q  -
```

```
       GTTACTCTGAAAGAGTCTGGCCCTGTACTAGTGAAGCCCACCGAGACCCTCACTCTGACT
   61  ---------+---------+---------+---------+---------+---------+ 120
        V  T  L  K  E  S  G  P  V  L  V  K  P  T  E  T  L  T  L  T   -

       TGTACTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGGGAGTGAGCTGGATTCGTCAG
  121  ---------+---------+---------+---------+---------+---------+ 180
        C  T  F  S  G  F  S  L  S  T  S  G  M  G  V  S  W  I  R  Q   -

       CCTCCAGGAAAGGCTCTGGAGTGGCTGGCACACATTTACTGGGATGATGACAAGCGCTAT
  181  ---------+---------+---------+---------+---------+---------+ 240
        P  P  G  K  A  L  E  W  L  A  H  I  Y  W  D  D  D  K  R  Y   -

       AACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAAAAGCCAGGTAGTC
  241  ---------+---------+---------+---------+---------+---------+ 300
        N  P  S  L  K  S  R  L  T  I  S  K  D  T  S  K  S  Q  V  V   -

       CTCACGATGACCAATATGGACCCTGTAGATACTGCCACATACTACTGTGTTCGAAGGCCC
  301  ---------+---------+---------+---------+---------+---------+ 360
        L  T  M  T  N  M  D  P  V  D  T  A  T  Y  Y  C  V  R  R  P   -

       ATTACTCCGGTACTAGTCGATGCTATGGACTACTGGGGCCAAGGAACCCTGGTCACCGTC
  361  ---------+---------+---------+---------+---------+---------+ 420
        I  T  P  V  L  V  D  A  M  D  Y  W  G  Q  G  T  L  V  T  V   -

       TCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC
  421  ---------+---------+---------+---------+---------+---------+ 480
        S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T   -

       TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
  481  ---------+---------+---------+---------+---------+---------+ 540
        S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T   -

       GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
  541  ---------+---------+---------+---------+---------+---------+ 600
        V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q   -

       TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
  601  ---------+---------+---------+---------+---------+---------+ 660
        S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T   -

       CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
  661  ---------+---------+---------+---------+---------+---------+ 720
        Q  T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V   -

       GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
  721  ---------+---------+---------+---------+---------+---------+ 780
        E  P  K  S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  L   -

       GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG
  781  ---------+---------+---------+---------+---------+---------+ 840
        G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R   -

       ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
  841  ---------+---------+---------+---------+---------+---------+ 900
        T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F   -

       AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
  901  ---------+---------+---------+---------+---------+---------+ 960
        N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q   -

       TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT
  961  ---------+---------+---------+---------+---------+---------+ 1020
        Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N   -

       GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
  1021 ---------+---------+---------+---------+---------+---------+ 1080
        G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T   -
```

```
                 ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG
           1081  ---------+---------+---------+---------+---------+---------+ 1140
                 I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  -

                 GATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
           1141  ---------+---------+---------+---------+---------+---------+ 1200
                 D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  -

                 GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
           1201  ---------+---------+---------+---------+---------+---------+ 1260
                 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  -

                 CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGC
           1261  ---------+---------+---------+---------+---------+---------+ 1320
                 P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  -

                 AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
           1321  ---------+---------+---------+---------+---------+---------+ 1380
                 R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  -

                 TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA        (SEQ ID NO:18)
           1381  ---------+---------+---------+------ 1416
                 Y  T  Q  K  S  L  S  L  S  P  G  K   -        (SEQ ID NO:20)
```

The complete sequence of a humanized 10D5 light chain gene with introns
(located between MluI and BamHI sites, as in pVk-Hu10D5) is shown below (SEQ ID
NO:15). The nucleotide number indicates its position in pVk-Hu10D5. The V_k and C_k
exons are translated in single letter code; the dot indicates the translation termination
codon. The mature light chain starts at the double-underlined aspartic acid (D). The
intron sequences are in italic. The expressed light chain corresponds to SEQ ID NO:11
when mature.

```
 619 ACGCGTCCACCATGAAGTTGCCTGTTAGGCTGTTGGTACTGATGTTCTGGATTCCTGCTTCCAGCAGTGATGTTGTGATG
                M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  V  V  M
 699 ACCCAATCTCCACTCTCCCTGCCTGTCACTCTTGGACAGCCAGCCTCCATCTCTTGCAGATCTAGTCAGAACATTATACA
      T  Q  S  P  L  S  L  P  V  T  L  G  Q  P  A  S  I  S  C  R  S  S  Q  N  I  I  H
 779 TAGTAATGGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTTCCA
      S  N  G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  R  L  L  I  Y  K  V  S
 859 ACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAG
      N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E
 939 GCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAAAT
      A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P  L  T  F  G  G  G  T  K  V  E  I
1019 AAAACGTAAGTGCACTTTCCTAATCTAGAAATTCTAAACTCTGAGGGGGTCGGATGACGTGGCCATTCTTTGCCTAAAGC
      K  R
1099 ATTGAGTTTACTGCAAGGTCAGAAAAGCATGCAAAGCCCTCAGAATGGCTGCAAAGAGCTCCAACAAAACAATTTAGAAC
1179 TTTATTAAGGAATAGGGGGAAGCTAGGAAGAAACTCAAAACATCAAGATTTTAAATACGCTTCTTGGTCTCCTTGCTATA
1259 ATTATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCA
1339 GAACTTTGTTACTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC
                                                       T  V  A  A  P  S  V  F  I  F  P
1419 CATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
      P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V
1499 CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTA
      Q  W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y
1579 CAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGG
      S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T  H  Q
1659 GCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCACCTGCTCCTCAGTTCC
      G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  •
1739 AGCCTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCTCATC
1819 TTTCACCTCACCCCCCTCCTCCTCCTTGGCTTTAATTATGCTAATGTTGGAGGAGAATGAATAAATAAA GTGAATCTTTG
```

```
1899 CACCTGTGGTTTCTCTCTTTCCTCATTTAATAATTATTATCTGTTGTTTTACCAACTACTCAATTTCTCTTATAAGGGAC
1979 TAAATATGTAGTCATCCTAAGGCGCATAACCATTTATAAAAATCATCCTTCATTCTATTTTACCCTATCATCCTCTGCAA
2059 GACAGTCCTCCCTCAAACCCACAAGCCTTCTGTCCTCACAGTCCCCTGGGCCATGGTAGGAGAGACTTGCTTCCTTGTTT
2139 TCCCCTCCTCAGCAAGCCCTCATAGTCCTTTTTAAGGGTGACAGGTCTTACAGTCATATATCCTTTGATTCAATTCCCTG
2219 AGAATCAACCAAAGCAAATTTTTCAAAAGAAGAAACCTGCTATAAAGAGAATCATTCATTGCAACATGATATAAAATAAC
2299 AACACAATAAAAGCAATTAAATAAACAAACAATAGGGAAATGTTTAAGTTCATCATGGTACTTAGACTTAATGGAATGTC
2379 ATGCCTTATTTACATTTTTAAACAGGTACTGAGGGACTCCTGTCTGCCAAGGGCCGTATTGAGTACTTTCCACAACCTAA
2459 TTTAATCCACACTATACTGTGAGATTAAAAACATTCATTAAAATGTTGCAAAGGTTCTATAAAGCTGAGAGACAAATATA
2539 TTCTATAACTCAGCAATCCCACTTCTAGGATCC          (SEQ ID NO:15)
```

The complete sequence of a humanized 10D5 heavy chain gene with introns (located between MluI and BamHI sites, as in pVg1-Hu10D5) is shown below (SEQ ID NO:16). The nucleotide number indicates its position in pVg1-Hu10D5. The V$_H$ and C$_H$ exons are translated in single letter code; the dot indicates the translation termination codon. The mature heavy chain starts at the double-underlined glutamine (Q). The intron sequences are in italic. The expressed heavy chain corresponds to SEQ ID NO:12 when mature.

```
619  ACGCGTCCACCATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCCTGCATATGTCCTGTCCCAGGTTACTCTG
           M  D  R  L  T  S  S  F  L  L  L  I  V  P  A  Y  V  L  S  Q  V  T  L
699  AAAGAGTCTGGCCCTGTACTAGTGAAGCCCACCGAGACCCTCACTCTGACTTGTACTTTCTCTGGGTTTTCACTGAGCAC
      K  E  S  G  P  V  L  V  K  P  T  E  T  L  T  L  T  C  T  F  S  G  F  S  L  S  T
779  TTCTGGTATGGGAGTGAGCTGGATTCGTCAGCCTCCAGGAAAGGCTCTGGAGTGGCTGGCACACATTTACTGGGATGATG
      S  G  M  G  V  S  W  I  R  Q  P  P  G  K  A  L  E  W  L  A  H  I  Y  W  D  D
859  ACAAGCGCTATAACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAAAAGCCAGGTAGTCCTCACGATG
      D  K  R  Y  N  P  S  L  K  S  R  L  T  I  S  K  D  T  S  K  S  Q  V  V  L  T  M
939  ACCAATATGGACCCTGTAGATACTGCCACATACTACTGTGTTCGAAGGCCCATTACTCCGGTACTAGTCGATGCTATGGA
      T  N  M  D  P  V  D  T  A  T  Y  Y  C  V  R  R  P  I  T  P  V  L  V  D  A  M  D
1019 CTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAGGTGAGTCCTCACAACCTCTAGAGCTTTCTGGGGCAGGCCAGG
      Y  W  G  Q  G  T  L  V  T  V  S  S
1099 CCTGACCTTGGCTTTGGGGCAGGGAGGGGGCTAAGGTGAGGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAG
1179 CCCAGACACTGGACGCTGAACCTCGCGGACAGTTAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACAC
1259 CGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC
                                           A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S
1339 ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGG
      T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G
1419 CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
      A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T
1499 TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAA
      V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K
1579 GTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCT
      V
1659 ATGCAGCCCCAGTCCAGGGCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTC
1739 AGGGAGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCCCTGCACACAA
1819 AGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGC
1899 CAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCC
                                                                                 .  E  P
1979 AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGA
      K  S  C  D  K  T  H  T  C  P  P  C  P
2059 CAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCA
                                                                                       A
2139 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
      P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E
2219 GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
      V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V
2299 ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
      H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q
2379 GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA
```

```
        D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K
 2459 AGCCAAAGGTGGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACC
        A  K
 2539 GCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGAC
                                   G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T
 2619 CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
        K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G
 2699 AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
        Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V
 2779 GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
        D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K
 2859 GAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGGCAAGCCCCCGCTCCCGGGCTCTCGCGGTCGCACGAGGA
        S  L  S  L  S  P  G  K  •
 2939 TGCTTGGCACGTACCCCCTGTACATACTTCCCGGGCGCCCAGCATGGAAATAAA  GCACCCAGCGCTGCCCTGGGCCCCTG
 3019 CGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGGCATGAGGGAGGCAGAGCGGGTCCCACT
 3099 GTCCCCACACTGGCCCAGGCTGTGCAGGTGTGCCTGGGCCGCCTAGGGTGGGGCTCAGCCAGGGGCTGCCCTCGGCAGGG
 3179 TGGGGGATTTGCCAGCGTGGCCCTCCCTCCAGCAGCACCTGCCCTGGGCTGGGCCACGGGAAGCCCTAGGAGCCCCTGGG
 3259 GACAGACACACAGCCCCTGCCTCTGTAGGAGACTGTCCTGTTCTGTGAGCGCCCCTGTCCTCCGACCTCCATGCCCACTCG
 3339 GGGGCATGCCTAGTCCATGTGCGTAGGGACAGGCCCTCCCTCACCCATCTACCCCTCACGGCACTAACCCCTGGCTGCCCT
 3419 GCCCAGCCTCGCACCCGCATGGGGACACAACCGACTCCGGGGACATGCACTCTCGGGCCCTGTGGAGGGACTGGTGCAGA
 3499 TGCCCACACACACACTCAGCCCAGACCCGTTCAACAAACCCCGCACTGAGGTTGGCCGGCCACACGGCCACCACACACAC
 3579 ACGTGCACGCCTCACACACGGAGCCTCACCCGGGCGAACTGCACAGCACCCAGACCAGAGCAAGGTCCTCGCACACGTGA
 3659 ACACTCCTCGGACACAGGCCCCCACGAGCCCCACGCGGCACCTCAAGGCCCACGAGCCTCTCGGCAGCTTCTCCACATGC
 3739 TGACCTGCTCAGACAAACCCAGCCCTCCTCTCACAAGGGTGCCCCTGCAGCCGCCACACACACACAGGGGATCACACACC
 3819 ACGTCACGTCCCTGGCCCTGGCCCACTTCCCAGTGCCGCCCTTCCCTGCAGGATCC          (SEQ ID NO:16)
```

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably from immortalized B-cells. Suitable source cells for the polynucleotide sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources well-known in the art.

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). These polypeptide fragments may be produced by proteolytic cleavage of intact antibodies by methods well known in the art, or by inserting stop codons at the desired locations in vectors using site-

directed mutagenesis, such as after CH1 to produce Fab fragments or after the hinge
region to produce F(ab')$_2$ fragments. Single chain antibodies may be produced by joining
VL and VH with a DNA linker.

As stated previously, the polynucleotides will be expressed in hosts after the

5   sequences have been operably linked to (i.e., positioned to ensure the functioning of) an
expression control sequence. These expression vectors are typically replicable in the host
organisms either as episomes or as an integral part of the host chromosomal DNA.
Commonly, expression vectors will contain selection markers, e.g., tetracycline or
neomycin, to permit detection of those cells transformed with the desired DNA

10   sequences.

E. coli is a prokaryotic host useful particularly for cloning the polynucleotides of
the present invention. Other microbial hosts suitable for use include bacilli, such as
Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various
Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors,

15   which will typically contain expression control sequences compatible with the host cell
(e.g., an origin of replication). In addition, any of a number of well-known promoters
may be present, such as the lactose promoter system, a tryptophan (trp) promoter system,
a beta-lactamase promoter system, or a promoter system from phage lambda. The
promoters will typically control expression, optionally with an operator sequence, and

20   have ribosome binding site sequences and the like, for initiating and completing
transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is
a preferred host, with suitable vectors having expression control sequences, such as
promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an

25   origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to
express and produce the polypeptides of the present invention. Eukaryotic cells are
actually preferred, because a number of suitable host cell lines capable of secreting intact
immunoglobulins have been developed in the art, and include the CHO cell lines, various

30   COS cell lines, Syrian Hamster Ovary cell lines, HeLa cells, preferably myeloma cell
lines, transformed B-cells, human embryonic kidney cell lines, or hybridomas.
Expression vectors for these cells can include expression control sequences, such as an

origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma

5   Virus, cytomegalovirus and the like.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic

10   cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Once expressed, the antibodies can be purified according to standard procedures, including ammonium sulfate precipitation, ion exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel electrophoresis, and the like.

15   Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or prophylactically, as directed herein.

The antibodies (including immunologically reactive fragments) are administered

20   to a subject at risk for or exhibiting Aβ-related symptoms or pathology such as clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical amyloid angiopathy, using standard administration techniques, preferably peripherally (*i.e.* not by administration into the central nervous system) by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or

25   suppository administration. Although the antibodies may be administered directly into the ventricular system, spinal fluid, or brain parenchyma, and techniques for addressing these locations are well known in the art, it is not necessary to utilize these more difficult procedures. The antibodies of the invention are effective when administered by the more simple techniques that rely on the peripheral circulation system. The advantages of the

30   present invention include the ability of the antibody to exert its beneficial effects even though not provided directly to the central nervous system itself. Indeed, it has been

demonstrated that the amount of antibody that crosses the blood-brain barrier is $\leq 0.1\%$ of plasma levels.

The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. <u>Remington's Pharmaceutical Sciences</u>, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners.

The concentration of the humanized antibody in formulations may range from as low as about 0.1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, and so forth, in accordance with the particular mode of administration selected. Thus, a pharmaceutical composition for injection could be made up to contain in 1 mL of phosphate buffered saline from 1 to 100 mg of the humanized antibody of the present invention. The formulation could be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250 mL of fluid, such as sterile Ringer's solution, and 1-100 mg per mL, or more in antibody concentration. Therapeutic agents of the invention can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 4 and 8 is tolerated.

Although the foregoing methods appear the most convenient and most appropriate for administration of proteins such as humanized antibodies, by suitable adaptation, other techniques for administration, such as transdermal administration and oral administration may be employed provided proper formulation is designed.

In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen.

In summary, formulations are available for administering the antibodies of the invention and are well-known in the art and may be chosen from a variety of options.

Typical dosage levels can be optimized using standard clinical techniques and will be dependent on the mode of administration and the condition of the patient.

5   The following examples are intended to illustrate but not to limit the invention.

The examples hereinbelow employ, among others, a murine monoclonal antibody designated "10D5" which was originally prepared by immunization with a peptide composed of residues 1-28 of human Aβ peptide. As the examples here describe experiments conducted in murine systems, the use of murine monoclonal antibodies is

10   satisfactory. However, in the treatment methods of the invention intended for human use, humanized forms of the antibodies with the immunospecificity corresponding to that of antibody 10D5 are preferred.

Example 1

15   Synthesis of Humanized Antibody 10D5

Cells and antibodies. Mouse myeloma cell line Sp2/0 was obtained from ATCC (Manassas, VA) and maintained in DME medium containing 10% FBS (Cat # SH30071.03, HyClone, Logan, UT) in a 37°C CO_2 incubator. Mouse 10D5 hybridoma cells were first grown in RPMI-1640 medium containing 10% FBS (HyClone), 10 mM

20   HEPES, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 25 µg/ml gentamicin, and then expanded in serum-free media (Hybridoma SFM, Cat # 12045-076, Life Technologies, Rockville, MD) containing 2% low Ig FBS (Cat # 30151.03, HyClone) to a 1.5 liter volume in roller bottles. Mouse monoclonal antibody 10D5 (Mu10D5) was purified from the culture supernatant by affinity chromatography

25   using a protein-G Sepharose column. Biotinylated Mu10D5 was prepared using EZ-Link Sulfo-NHS-LC-LC-Biotin (Cat # 21338ZZ, Pierce, Rockford, IL).

Cloning of variable region cDNAs. Total RNA was extracted from approximately 10^7 hybridoma cells using TRIzol reagent (Cat. # 15596-026, Life Technologies) and poly(A)$^+$ RNA was isolated with the PolyATract mRNA Isolation System (Cat. # Z5310,

30   Promega, Madison, WI) according to the suppliers' protocols. Double-stranded cDNA was synthesized using the SMART™RACE cDNA Amplification Kit (Cat. # K1811-1, Clontech, Palo Alto, CA) following the supplier's protocol. The variable region cDNAs

for the light and heavy chains were amplified by polymerase chain reaction (PCR) using 3'

primers that anneal respectively to the mouse kappa and gamma chain constant regions,

and a 5' universal primer provided in the SMART™RACE cDNA Amplification Kit. For

VL PCR, the 3' primer has the sequence:

5

5'-TATAGAGCTCAAGCTTGGATGGTGGGAAGATGGATACAGTTGGTGC-3'

[SEQ ID NO:13]

with residues 17- 46 hybridizing to the mouse Ck region. For VH PCR, the 3' primers

10   have the degenerate sequences:

```
                                    A          G      T
5'-TATAGAGCTCAAGCTTCCAGTGGATAGACCGATGGGGCTGTCGTTTTGGC-3'
                                    T
```

15   [SEQ ID NO:14]

with residues 17 - 50 hybridizing to mouse gamma chain CH1. The VL and VH cDNAs

were subcloned into pCR4Blunt-TOPO vector (Cat. # 45-0031, Invitrogen, Carlsbad, CA)

for sequence determination. DNA sequencing was carried out by PCR cycle sequencing

20   reactions with fluorescent dideoxy chain terminators (Applied Biosystems, Foster City,

CA) according to the manufacturer's instructions. The sequencing reactions were

analyzed on a Model 377 DNA Sequencer (Applied Biosystems).

Construction of humanized 10D5 (Hu10D5) variable regions. Humanization of
the mouse antibody V regions was carried out as outlined by Queen et al., 1989, *op. Cit.*
The human V region framework used as acceptor for Mu10D5 CDRs was chosen based
on sequence homology. The computer programs ABMOD and ENCAD [Levitt, M., J.

5   Mol. Biol. 168:595-620 (1983)] were used to construct a molecular model of the variable
regions. Amino acids in the humanized V regions that were predicted to have contact
with CDRs were substituted with the corresponding residues of Mu10D5. This was done
at residue 98 in the heavy chain and at residues 41 and 51 in the light chain. The amino
acids in the humanized V region that were found to be rare in the same V-region subgroup

10   were changed to the consensus amino acids to eliminate potential immunogenicity. This
was done at residues 42 and 44 in the light chain and at residue 24 in the heavy chain.

The light and heavy chain variable region genes were constructed and amplified
using eight overlapping synthetic oligonucleotides ranging in length from approximately
65 to 80 bases [He, X. Y., et al., J. Immunol. 160: 1029-1035 (1998)]. The

15   oligonucleotides were annealed pairwise and extended with the Klenow fragment of DNA
polymerase I, yielding four double-stranded fragments. The resulting fragments were
denatured, annealed pairwise, and extended with Klenow, yielding two fragments. These
fragments were denatured, annealed pairwise, and extended once again, yielding a full-
length gene. The resulting product was amplified by PCR using the Expand High Fidelity

20   PCR System (Cat. # 1 732 650, Roche Molecular Biochemicals, Indianapolis, IN). The
PCR-amplified fragments were gel-purified and cloned into pCR4Blunt-TOPO vector.
After sequence confirmation, the VL and VH genes were digested with MluI and XbaI,
gel-purified, and subcloned respectively into vectors for expression of light and heavy
chains to make pVk-Hu10D5 and pVg1-Hu10D5 [Co, M. S., et al., J. Immunol.

25   148:1149-1154 (1992)]. The mature humanized 10D5 antibody expressed from these
plasmids has the light chain of SEQ ID NO:11 and the heavy chain of SEQ ID NO:12.

Stable transfection. Stable transfection into mouse myeloma cell line Sp2/0 was
accomplished by electroporation using a Gene Pulser apparatus (BioRad, Hercules, CA)
at 360 V and 25 µF as described (Co, et al., 1992, *op. cit.*). Before transfection, pVk-

30   Hu10D5 and pVg1-Hu10D5 plasmid DNAs were linearized using FspI. Approximately
10^7 Sp2/0 cells were transfected with 20 µg of pVk-Hu10D5 and 40 µg of pVg1-Hu10D5.
The transfected cells were suspended in DME medium containing 10% FBS and plated

into several 96-well plates. After 48 hr, selection media (DME medium containing 10% FBS, HT media supplement, 0.3 mg/ml xanthine and 1 µg/ml mycophenolic acid) was applied. Approximately 10 days after the initiation of the selection, culture supernatants were assayed for antibody production by ELISA as shown below. High yielding clones

5   were expanded in DME medium containing 10% FBS and further analyzed for antibody expression. Selected clones were then adapted to growth in Hybridoma SFM.

Measurement of antibody expression by ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc, Naperville, IL) were coated with 100 µl of 1 µg/ml goat anti-human IgG, Fc γ fragment specific, polyclonal antibodies (Cat. #

10   109-005-098, Jackson ImmunoResearch, West Grove, PA) in 0.2 M sodium carbonate-bicarbonate buffer (pH 9.4) overnight at 4°C. After washing with Washing Buffer (PBS containing 0.1% Tween 20), wells were blocked with 400 µl of Superblock Blocking Buffer (Cat # 37535, Pierce) for 30 min and then washed with Washing Buffer. Samples containing Hu10D5 were appropriately diluted in ELISA Buffer (PBS containing 1%

15   BSA and 0.1% Tween 20) and applied to ELISA plates (100 µl per well). As a standard, humanized anti-CD33 IgG1 monoclonal antibody HuM195 (Co, et al., 1992, op. cit.) was used. The ELISA plate was incubated for 2 hr at room temperature and the wells were washed with Washing Buffer. Then, 100 µl of 1/1,000-diluted HRP-conjugated goat anti-human kappa polyclonal antibodies (Cat # 1050-05, Southern Biotechnology,

20   Birmingham, AL) in ELISA Buffer was applied to each well. After incubating for 1 hr at room temperature and washing with Washing Buffer, 100 µl of ABTS substrate (Cat #s 507602 and 506502, Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. Color development was stopped by adding 100 µl of 2% oxalic acid per well. Absorbance was read at 415 nm using an OPTImax microplate reader (Molecular

25   Devices, Menlo Park, CA).

Purification of Hu10D5. One of the high Hu10D5-expressing Sp2/0 stable transfectants (clone #1) was adapted to growth in Hybridoma SFM and expanded to 2 liters in roller bottles. Spent culture supernatant was harvested when cell viability reached 10% or below and loaded onto a protein-A Sepharose column. The column was

30   washed with PBS before the antibody was eluted with 0.1 M glycine-HCl (pH 2.8), 0.1 M NaCl. The eluted protein was dialyzed against 3 changes of 2 liters of PBS and filtered through a 0.2 µm filter prior to storage at 4°C. Antibody concentration was determined

by measuring absorbance at 280 nm (1 mg/ml = 1.4 A_{280}). SDS-PAGE in Tris-glycine
buffer was performed according to standard procedures on a 4-20% gradient gel (Cat #
EC6025, Novex, San Diego, CA). Purified humanized 10D5 antibody is reduced and run
on an SDS- PAGE gel. The whole antibody shows two bands of approximate molecular
weights 25 kDa and 50 kDa. These results are consistent with the molecular weights of
the light chain and heavy chain, or with the molecular weight of the chain(s) comprising a
fragment, calculated from their amino acid compositions.

Example 2
In vitro binding properties of humanized 10D5 antibody

The binding efficacy of humanized 10D5 antibody, synthesized and purified as
described above, was compared with the mouse 10D5 antibody using biotinylated mouse
10D5 antibody in a comparative ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno
plate, Cat # 439454, NalgeNunc) were coated with 100 µl of β-amyloid peptide (1-42) in
0.2 M sodium carbonate/bicarbonate buffer (pH 9.4) (1 µg/mL) overnight at 4°C.

After washing the wells with phosphate buffered saline (PBS) containing 0.1%
Tween 20 (Washing Buffer) using an ELISA plate washer, the wells were blocked by
adding 300 µL of SuperBlock reagent (Pierce) per well. After 30 minutes of blocking, the
wells were washed with Washing Buffer and excess liquid was removed.

A mixture of biotinylated Mu10D5 (0.4 µg/ml final concentration) and competitor
antibody (Mu10D5 or Hu10D5; starting at 1000 µg/ml final concentration and serial 3-
fold dilutions) in ELISA Buffer were added in triplicate in a final volume of 100 µl per
well. As a no-competitor control, 100 µl of 0.4 µg/ml biotinylated Mu10D5 was added.
As a background control, 100 µl of ELISA Buffer was added. The ELISA plate was
incubated at room temperature for 90 min. After washing the wells with Washing Buffer,
100 µl of 10 µg/ml HRP-conjugated streptavidin (Cat # 21124, Pierce) was added to each
well. The plate was incubated at room temperature for 30 min and washed with Washing
Buffer. For color development, 100 µl/well of ABTS Peroxidase Substrate (Kirkegaard &
Perry Laboratories) was added. Color development was stopped by adding 100 µl/well of
2% oxalic acid. Absorbance was read at 415 nm. The absorbances were plotted against
the log of the competitor concentration, curves were fit to the data points (using Prism)
and the IC50 was determined for each antibody using methods well-known in the art.

The mean IC50 for mouse 10D5 was 23.4 µg/mL (three separate experiments,

standard deviation =5.5 µg/mL) and for humanized 10D5 was 49.1 µg/mL (three separate

experiments, standard deviation = 11.8 µg/mL). A second set of three experiments was

carried out, essentially as described above, and the mean IC50 for mouse 10D5 was

5   determined to be 20 µg/mL (SD = 1 µg/mL) and for humanized 10D5, the IC50 was

determined to be 16 µg/mL (SD = 0.6 µg/mL). On the basis of these results, we conclude

that humanized 10D5 has binding properties that are very similar to those of the mouse

antibody 10D5. Therefore, we expect that humanized 10D5 has very similar *in vitro* and

in vivo activities compared with mouse 10D5 and will exhibit in humans the same effects

10   demonstrated with mouse 10D5 in mice.

Example 3

In vitro binding properties of mouse and humanized antibodies 10D5

Antibody affinity (KD = Kd / Ka) was determined using a BIAcore biosensor 2000

15   and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-

mouse Ig or anti-human Ig) was coupled via free amine groups to carboxyl groups on flow

cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and

N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG was coupled to flow cell

1 as a background control. Monoclonal antibodies were captured to yield 300 resonance

20   units (RU). Amyloid-beta 1-40 or 1-42 (Biosource International, Inc.) was then flowed

over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the

chip, bound anti-Aβ antibody was eluted from the chip using a wash with glycine-HCl

(pH 2). A control injection containing no amyloid-beta served as a control for baseline

subtraction. Sensorgrams demonstrating association and dissociation phases were

25   analyzed to determine Kd and Ka. The affinity (KD) of mouse antibody 10D5 for Aβ 1-

40 was determined to be 390 nM, and the affinity of humanized 10D5, prepared

essentially as described in Example 1, was determined to be 209 nM. Affinity for Aβ 1-

42 was biphasic for both mouse 10D5 and humanized 10D5. For mouse 10D5, the

affinities for Aβ 1-42 were 0.57 nM and 4950 nM. Humanized 10D5 had affinities for

30   Aβ 1-42 of 0.19 nM and 1020 nM.

Example 4

Epitope mapping of mouse and humanized 10D5

The BIAcore is an automated biosensor system for measuring molecular
interactions [Karlsson R., et al. J. Immunol. Methods 145:229-240 (1991)]. The

5   advantage of the BIAcore over other binding assays is that binding of the antigen can be
measured without having to label or immobilize the antigen (i.e. the antigen maintains a
more native conformation).   The BIAcore methodology was used to assess the binding of
various amyloid-beta peptide fragments to either mouse 10D5 or humanized 10D5
(prepared substantially as described in Example 1). All dilutions were made with HEPES

10   buffered saline containing Tween 20. A single concentration of a variety of fragments of
human $A\beta$ or mouse $A\beta$ 1-40 (BioSource International) was used. Human amyloid beta
fragments 1-10 and 1-20 bound to mouse 10D5 and to humanized 10D5, while human $A\beta$
fragments 10-20 and 16-25 did not bind to either antibody. Neither mouse 10D5 nor
humanized 10D5 bound mouse $A\beta$ 1-40. Using this methodology, the binding epitope for

15   both mouse and humanized 10D5 appears to be between amino acids 1 and 10 of human
$A\beta$.

Example 5

In vivo experiments with 10D5

Unless otherwise stated, all studies used PDAPP mice, and all injections were

20   intraperitoneal (i.p.) In general, a control group of mice received injections of saline. In
some cases, another control group received injections of a non-specific mouse IgG
preparation.

Six weeks of weekly injection of 360 µg of 10D5 in old mice (24 month) raised
soluble $A\beta_{total}$ in hippocampus by 16% and $A\beta$ 1-42 in hippocampus by 21%, while

25   lowering hippocampal insoluble $A\beta_{total}$ by 24% and $A\beta$ 1-42 by 26% (no statistically
significant difference; 9 animals per control group and 10 animals per antibody group). In
the cortex, mean insoluble $A\beta_{total}$ was lower by 27% and $A\beta$ 1-42 by 29%, while mean
insoluble $A\beta$ 1-40 increased by 7% (no statistically significant differences).

In hemizygous, 4 month old mice, administration of 360 µg 10D5 per animal: 1)

30   raised average plasma $A\beta$ 1-40 and $A\beta$ 1-42 levels approximately 3-fold by 24 hours after
administration; and 2) had no significant effect on soluble $A\beta$ 1-40 in the cortex after 24

hours compared with saline control (no differences were statistically significant; 5 animals per group).

Administration of 360 µg of 10D5 per animal (5 animals per group, saline control): 1) raised average plasma Aβ 1-40 and Aβ 1-42 levels approximately 14-fold and 19-fold, respectively by 24 hours after administration; 2) had no consistent or significant effect on soluble or insoluble Aβ 1-40, Aβ 1-42, or Aβ_{total} in the cortex or hippocampus after 24 hours; 3) lowered soluble Aβ 1-40, Aβ 1-42, and Aβ_{total} in the cerebellum by 50% ($p<0.05$), 33%, and 13%, respectively; and 4) lowered insoluble Aβ 1-40, Aβ 1-42, and Aβ_{total} in the cerebellum by 53% ($p<0.001$), 46% ($p<0.001$), and 30% ($p<0.01$), respectively.

In young mice, administration of 360 µg of 10D5 per animal (5 per group): 1) raised average plasma Aβ 1-42 levels approximately 33% by 24 hours after administration; and 2) in the cortex, raised soluble Aβ 1-40 3.4-fold ($p<0.001$), lowered soluble Aβ 1-42 by 22% ($p<0.05$), lowered insoluble Aβ 1-40 about 10% and increased insoluble Aβ 1-42 about 12%.

We claim:

1.   Humanized 10D5 antibody.

2.   A humanized antibody, or fragment thereof, comprising a humanized light chain comprising three light chain complementarity determining regions (CDRs) from the mouse monoclonal antibody 10D5 and a light chain variable region framework sequence from a human immunoglobulin light chain; and a humanized heavy chain comprising three heavy chain CDRs from the mouse monoclonal antibody 10D5 and a heavy chain variable region framework sequence from a human immunoglobulin heavy chain; wherein the light chain CDRs have the following amino acid sequences:

light chain CDR1:
```
1                5                       10                      15
Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)
```

light chain CDR2:
```
1                5
Lys Val Ser Asn Arg Phe Ser   (SEQ ID NO:2)
```

light chain CDR3:
```
1                5
Phe Gln Gly Ser His Val Pro Leu Thr   (SEQ ID NO:3)
```

and the heavy chain CDRs have the following amino acid sequences:

heavy chain CDR1:
```
1                5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)
```

heavy chain CDR2:
```
1                5                       10                      15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)
```

and, heavy chain CDR3:
```
1                5                       10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr   (SEQ ID NO:6).
```

3.   A humanized antibody or fragment thereof comprising a humanized light chain variable region having the sequence of SEQ ID NO:7 and a humanized heavy variable region having the sequence of SEQ ID NO:8.

4.   The humanized antibody or fragment thereof of claim 3 having a light chain variable region of the sequence given by SEQ ID NO:9 and a heavy chain variable region given by SEQ ID NO:10.

5.   The humanized antibody or fragment thereof of claim 3 having a light chain of the sequence given by SEQ ID NO:11 and a heavy chain of the sequence given by SEQ ID NO:12.

6.   An antibody fragment obtainable by enzymatic cleavage of the humanized antibody of any one of claims 1 - 5.

7.   An Fab or F(ab')$_2$ fragment of any one of the humanized antibodies of claims 1 - 5.

8.   The F(ab')$_2$ fragment of claim 7.

9.   The Fab fragment of claim 7.

10.   The humanized antibody or fragment of any one of claims 1 – 9, which is a single chain antibody.

11.   The humanized antibody or fragment of any one of claims 1 - 10 that is an IgG$_1$ immunoglobulin isotype.

12.   The humanized antibody or fragment of any one of claims 1 - 11, wherein the antibody or fragment thereof is produced in a host cell selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

13.   A polynucleotide compound, comprising a sequence coding for the light chain or the heavy chain of the humanized antibody of any one of claims 1 - 12, or a fragment thereof.

14.   A polynucleotide sequence, which when expressed in a suitable host cell, yields an antibody of any one of claims 1 – 12.

15.   The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 17, and a polynucleotide comprising a sequence that codes for the light chain variable region given by SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO: 11.

16.   The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:18, and a polynucleotide comprising a sequence that codes for the heavy chain variable region given by SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:12.

17.   An expression vector for expressing the antibody of any one of claims 1 - 12 comprising the polynucleotide sequence of any one of claims 13 - 16.

18.   A cell transfected with the expression vector of claim 17.

19.   A cell transfected with two expression vectors of claim 17, wherein a first vector comprises the polynucleotide sequence coding for the light chain and a second vector comprises the sequence coding for the heavy chain.

20.   A cell that is capable of expressing the humanized antibody or fragment of any one of claims 1 – 12.

21.   The cell of any one of claims 18 – 20, wherein the cell is selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

22.   A pharmaceutical composition comprising the humanized antibody or fragment of any one of claims 1 – 12, and a pharmaceutically acceptable excipient.

23. A method of treating Down s synurome, clinical or pre-clinical Alzheimer's disease, or clinical or pre-clinical cerebral amyloid angiopathy in a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

24. A method to inhibit the formation of Aβ plaque in the brain of a human subject, comprising administering to the human subject an effective amount of the humanized antibody or fragment of any one of claims 1 – 12.

25. A method to reduce Aβ plaque in the brain of a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

26. The method of either of claims 24 – 25, wherein the subject is diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

27. The method of any one of claims 24 – 25, wherein the subject is not diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

28. Use of the humanized antibody or a fragment thereof according to any one of Claims 1 – 12 for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

29. Use of the humanized antibody or fragment of any one of claims 1 – 12 for the manufacture of a medicament for treating Alzheimer's disease.

SEQUENCE LISTING

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<151>   2001-04-30

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             20   25   30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
             35   40   45

Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
     50   55   60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65   70   75   80

Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Phe Gln Gly
                 85   90   95

Ser His Val Pro Leu Thr Phe Gly Xaa Gly Thr Lys Xaa Glu Ile Lys
                 100   105   110

Arg

<210> 8
<211> 123
<212> PRT
<213> humanized antibody

<220>
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<223> Xaa=Gln or Glu

<220>
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<222> (2)..(2)
<223> Xaa=Val or Ala

<220>
<221> MISC_FEATURE
<222> (64)..(64)
<223> Xaa=Ser or Thr

<220>
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<222> (77)..(77)
<223> Xaa=Lys or Arg

<220>
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<222> (78)..(78)
<223> Xaa=Ser or Thr

<220>

<221>   MISC_FEATURE

<222>   (83)..(83)

<223>   Xaa=Thr or Ser

<220>

<221>   MISC_FEATURE

<222>   (84)..(84)

<223>   Xaa=Met, Ile or Leu

<220>

<221>   MISC_FEATURE

<222>   (86)..(86)

<223>   Xaa=Asn, Ser, Thr

<220>

<221>   MISC_FEATURE

<222>   (87)..(87)

<223>   Xaa=Met, Val, or Leu

<220>

<221>   MISC_FEATURE

<222>   (118)..(118)

<223>   Xaa=Leu or Ser

<400>   8

Xaa Xaa Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1   5   10   15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20   25   30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35   40   45

```
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Xaa
    50              55              60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val
65              70              75              80

Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr
            85              90              95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
            100             105             110

Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
            115             120
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<210> 9

<211> 113

<212> PRT

<213> humanized antibody

<400> 9

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Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5               10              15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
            20              25              30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
            35              40              45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50              55              60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65              70              75              80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85              90              95

Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100             105             110

Arg
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<210> 10
<211> 123
<212> PRT
<213> humanized antibody

<400> 10

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1   5   10   15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
             20   25   30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
         35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
     50   55   60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
65   70   75   80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                 85   90   95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
             100   105   110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
         115   120

<210> 11
<211> 219
<212> PRT
<213> humanized antibody

<400> 11

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1   5   10   15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
             20   25   30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
        50   55   60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85   90   95

Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105   110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115   120   125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130   135   140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145   150   155   160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165   170   175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180   185   190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
            195   200   205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210   215

<210>   12

<211>   453

<212>   PRT

<213>   humanized antibody

<400>   12

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1   5   10   15

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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20              25              30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35              40              45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
        50              55              60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
65              70              75              80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
            85              90              95

Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
            100             105             110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115             120             125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130             135             140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145             150             155             160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
            165             170             175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180             185             190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195             200             205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210             215             220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225             230             235             240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            245             250             255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260             265             270
```

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Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275             280             285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290             295             300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305             310             315             320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325             330             335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340             345             350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355             360             365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370             375             380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385             390             395             400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            405             410             415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420             425             430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435             440             445

Leu Ser Pro Gly Lys
        450
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<210> 13

<211> 46

<212> DNA

<213> DNA primer

<400> 13
tatagagctc aagcttggat ggtgggaaga tggatacagt tggtgc   46

<210> 14

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<211>    50

<212>    DNA

<213>    DNA primer


<400>    14
tatagagctc aagcttccag tggatagach gatggggstg tygttttggc              50

<210>    15

<211>    1953

<212>    DNA

<213>    humanized antibody


<400>    15
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ccagcagtga tgttgtgatg acccaatctc cactctccct gcctgtcact cttggacagc    120

cagcctccat ctcttgcaga tctagtcaga acattataca tagtaatgga aacacctatt    180

tagaatggta cctgcagaaa ccaggccagt ctccaaggct cctgatctac aaagtttcca    240

accgattttc tggggtccca gacaggttca gtggcagtgg atcagggaca gatttcacac    300

tcaagatcag cagagtggag gctgaggatg tgggagttta ttactgcttt caaggttcac    360

atgttccgct cactttcggc ggagggacca aggtggaaat aaaacgtaag tgcactttcc    420

taatctagaa attctaaact ctgagggggt cggatgacgt ggccattctt tgcctaaagc    480

attgagttta ctgcaaggtc agaaaagcat gcaaagccct cagaatggct gcaaagagct    540

ccaacaaaac aatttagaac tttattaagg aataggggga agctaggaag aaactcaaaa    600

catcaagatt ttaaatacgc ttcttggtct ccttgctata attatctggg ataagcatgc    660

tgttttctgt ctgtccctaa catgccctgt gattatccgc aaacaacaca cccaagggca    720

gaactttgtt acttaaacac catcctgttt gcttctttcc tcaggaactg tggctgcacc    780

atctgtcttc atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt    840

gtgcctgctg ataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc    900

cctccaatcg ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta    960

cagcctcagc agcaccctga cgctgagcaa agcagactac gagaaacaca agtctacgc    1020

ctgcgaagtc acccatcagg gcctgagctc gcccgtcaca aagagcttca cagggggaga    1080

gtgttagagg gagaagtgcc cccacctgct cctcagttcc agcctgaccc cctcccatcc    1140

tttggcctct gacccttttt ccacagggga cctacccccta ttgcggtcct ccagctcatc    1200

tttcacctca ccccctcct cctccttggc tttaattatg ctaatgttgg aggagaatga    1260
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ataaataaag tgaatctttg cacctgtggt ttctctcttt cctcatttaa taattattat    1320
ctgttgtttt accaactact caatttctct tataagggac taaatatgta gtcatcctaa    1380
ggcgcataac catttataaa aatcatcctt cattctattt taccctatca tcctctgcaa    1440
gacagtcctc cctcaaaccc acaagccttc tgtcctcaca gtccctgggg ccatggtagg    1500
agagacttgc ttccttgttt tcccctcctc agcaagccct catagtcctt tttaagggtg    1560
acaggtctta cagtcatata tcctttgatt caattccctg agaatcaacc aaagcaaatt    1620
tttcaaaaga agaaacctgc tataaagaga atcattcatt gcaacatgat ataaaataac    1680
aacacaataa aagcaattaa ataaacaaac aatagggaaa tgtttaagtt catcatggta    1740
cttagactta atggaatgtc atgccttatt tacatttta aacaggtact gagggactcc     1800
tgtctgccaa gggccgtatt gagtactttc cacaacctaa tttaatccac actatactgt    1860
gagattaaaa acattcatta aaatgttgca aaggttctat aaagctgaga gacaaatata    1920
ttctataact cagcaatccc acttctagga tcc                                 1953
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<210>  16
<211>  3256
<212>  DNA
<213>  humanized antibody

<400>  16
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tcactctgac ttgtactttc tctgggtttt cactgagcac ttctggtatg ggagtgagct     180
ggattcgtca gcctccagga aaggctctgg agtggctggc acacatttac tgggatgatg     240
acaagcgcta taacccatcc ctgaagagcc ggctcacaat ctccaaggat acctccaaaa     300
gccaggtagt cctcacgatg accaatatgg accctgtaga tactgccaca tactactgtg     360
ttcgaaggcc cattactccg gtactagtcg atgctatgga ctactggggc caaggaaccc     420
tggtcaccgt ctcctcaggt gagtcctcac aacctctaga gctttctggg gcaggccagg     480
cctgaccttg gctttggggc agggaggggg ctaaggtgag gcaggtggcg ccagccaggt     540
gcacacccaa tgcccatgag cccagacact ggacgctgaa cctcgcggac agttaagaac     600
ccaggggcct ctgcgccctg gcccagctc tgtcccacac cgcggtcaca tggcaccacc     660
tctcttgcag cctccaccaa gggcccatcg gtcttccccc tggcaccctc tccaagagc     720
acctctgggg gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg     780
acggtgtcgt ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta     840
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cagtcctcag gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc    900

acccagacct acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaaa    960

gttggtgaga ggccagcaca gggagggagg gtgtctgctg gaagccaggc tcagcgctcc   1020

tgcctggacg catcccggct atgcagcccc agtccagggc agcaaggcag gccccgtctg   1080

cctcttcacc cggaggcctc tgcccgcccc actcatgctc agggagaggg tcttctggct   1140

ttttccccag gctctgggca ggcacaggct aggtgcccct aacccaggcc ctgcacacaa   1200

aggggcaggt gctgggctca gacctgccaa gagccatatc cgggaggacc ctgcccctga   1260

cctaagccca ccccaaaggc caaactctcc actccctcag ctcggacacc ttctctcctc   1320

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cacatgccca ccgtgcccag gtaagccagc ccaggcctcg ccctccagct caaggcggga   1440

caggtgccct agagtagcct gcatccaggg acaggcccca gccgggtgct gacacgtcca   1500

cctccatctc ttcctcagca cctgaactcc tggggggacc gtcagtcttc ctcttccccc   1560

caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc gtggtggtgg   1620

acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc gtggaggtgc   1680

ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt gtggtcagcg   1740

tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc aaggtctcca   1800

acaaagccct cccagccccc atcgagaaaa ccatctccaa agccaaaggt gggacccgtg   1860

gggtgcgagg gccacatgga cagaggccgg ctcggcccac cctctgccct gagagtgacc   1920

gctgtaccaa cctctgtccc tacagggcag ccccgagaac cacaggtgta caccctgccc   1980

ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggcttc   2040

tatcccagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag   2100

accacgcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcaa gctcaccgtg   2160

gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg   2220

cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtaaatgagt gcgacggccg   2280

gcaagccccc gctccccggg ctctcgcggt cgcacgagga tgcttggcac gtaccccctg   2340

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cgagactgtg atggttcttt ccacgggtca ggccgagtct gaggcctgag tggcatgagg   2460

gaggcagagc gggtcccact gtccccacac tggcccaggc tgtgcaggtg tgcctgggcc   2520

gcctagggtg gggctcagcc aggggctgcc ctcggcaggg tgggggattt gccagcgtgg   2580

ccctccctcc agcagcacct gccctgggct gggccacggg aagccctagg agccctggg    2640

gacagacaca cagcccctgc ctctgtagga gactgtcctg ttctgtgagc gccctgtcct   2700

ccgacctcca tgcccactcg ggggcatgcc tagtccatgt gcgtagggac aggccctccc   2760
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tcacccatct accccacgg cactaacccc tggctgccct gcccagcctc gcacccgcat    2820

ggggacacaa ccgactccgg ggacatgcac tctcgggccc tgtggaggga ctggtgcaga    2880

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cacacggcca ccacacacac acgtgcacgc ctcacacacg gagcctcacc cgggcgaact    3000

gcacagcacc cagaccagag caaggtcctc gcacacgtga acactcctcg gacacaggcc    3060

cccacgagcc ccacgcggca cctcaaggcc cacgagcctc tcggcagctt ctccacatgc    3120

tgacctgctc agacaaaccc agccctcctc tcacaagggt gcccctgcag ccgccacaca    3180

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cttccctgca ggatcc                                                    3256
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<210> 17
<211> 714
<212> DNA
<213> humanized antibody

<400> 17
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gttgtgatga cccaatctcc actctccctg cctgtcactc ttggacagcc agcctccatc     120

tcttgcagat ctagtcagaa cattatacat agtaatggaa acacctattt agaatggtac     180

ctgcagaaac caggccagtc tccaaggctc ctgatctaca agtttccaa ccgattttct      240

ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc     300

agagtggagg ctgaggatgt gggagtttat tactgctttc aaggttcaca tgttccgctc     360

actttcggcg gagggaccaa ggtggaaata aaacgaactg tggctgcacc atctgtcttc     420

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg     480

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg     540

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc     600

agcaccctga cgctgagcaa agcagactac gagaaacaca agtctacgc ctgcgaagtc      660

acccatcagg gcctgagctc gcccgtcaca aagagcttca caggggaga gtgt            714
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<210> 18
<211> 1416
<212> DNA
<213> humanized antibody

<400> 18
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tgtactttct ctgggttttc actgagcact tctggtatgg gagtgagctg gattcgtcag   180

cctccaggaa aggctctgga gtggctggca cacatttact gggatgatga caagcgctat   240

aacccatccc tgaagagccg gctcacaatc tccaaggata cctccaaaag ccaggtagtc   300

ctcacgatga ccaatatgga ccctgtagat actgccacat actactgtgt tcgaaggccc   360

attactccgg tactagtcga tgctatggac tactggggcc aaggaaccct ggtcaccgtc   420

tcctcagcct ccaccaaggg cccatcggtc ttccccctgg caccctcctc aagagcacc   480

tctgggggca gcggccct gggctgcctg gtcaaggact acttccccga accggtgacg   540

gtgtcgtgga actcaggcgc cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag   600

tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcag cttgggcacc   660

cagacctaca tctgcaacgt gaatcacaag cccagcaaca ccaaggtgga caagaaagtt   720

gagcccaaat cttgtgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg   780

gggggaccgt cagtcttcct cttccccccca aaacccaagg acaccctcat gatctcccgg   840

accctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc   900

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag   960

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat   1020

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagcccccat cgagaaaacc   1080

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg   1140

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatcccagc   1200

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct   1260

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc   1320

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac   1380

tacacgcaga agagcctctc cctgtctccg ggtaaa   1416

<210> 19

<211> 238

<212> PRT

<213> humanized antibody

<400> 19

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1   5   10   15

Ser Ser Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
            20   25   30

Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile
        35   40   45

Ile His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
    50   55   60

Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65   70   75   80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
            85   90   95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
            100   105   110

Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val
        115   120   125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130   135   140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145   150   155   160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
            165   170   175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
        180   185   190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195   200   205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210   215   220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225   230   235

<210>   20

<211>   472

<212> PRT

<213> humanized antibody

<400> 20

Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
1   5   10   15

Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys
            20   25   30

Pro Thr Glu Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
            35   40   45

Ser Thr Ser Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
        50   55   60

Ala Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
65   70   75   80

Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys
                85   90   95

Ser Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala
            100   105   110

Thr Tyr Tyr Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala
            115   120   125

Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
        130   135   140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145   150   155   160

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
                165   170   175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
            180   185   190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
        195   200   205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
    210   215   220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225   230   235   240

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
                245   250   255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
              260   265   270

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
         275   280   285

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    290   295   300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305   310   315   320

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
              325   330   335

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
         340   345   350

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
    355   360   365

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
370   375   380

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385   390   395   400

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
              405   410   415

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
         420   425   430

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
    435   440   445

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    450   455   460

Ser Leu Ser Leu Ser Pro Gly Lys
465   470